Nutritional Analysis of Distillers Grains Fermented with Saccharomyces cerevisiae and Evaluation of Its Application Value in Swine Feed: Postprint

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Abstract

To evaluate the application value of distiller' s grains cultured with Saccharomyces cerevisiae in pig feed, this experiment determined nutritional components including dry matter, crude protein, crude fat, crude ash, crude fiber, and amino acid contents in the cultured product, and subsequently measured the apparent total tract digestibility of nutrients in pigs using the total fecal collection method, as well as the apparent ileal digestibility of amino acids through T-cannula installation. The results indicated that distiller' s grains cultured with Saccharomyces cerevisiae contained 8.34% moisture, 31.12% crude protein, 5.00% crude fat, 13.46% crude ash, and 11.00% crude fiber, with a gross energy content of 19.53 MJ/kg, and the mycotoxin content was far below the national standard. The apparent total tract digestibility of dry matter, gross energy, crude protein, crude fat, crude fiber, and crude ash in distiller' s grains cultured with Saccharomyces cerevisiae for pigs was 48.79%, 38.33%, 54.15%, 82.94%, 23.17%, and 20.29%, respectively, with a digestible energy value of 11.75 MJ/kg. The average apparent ileal digestibility of essential and non-essential amino acids in distiller' s grains cultured with Saccharomyces cerevisiae for pigs was 72.38% and 69.37%, respectively, with the apparent ileal digestibility of both lysine and methionine exceeding 80%. It was concluded that the absorption and utilization efficiency of amino acids in distiller' s grains cultured with Saccharomyces cerevisiae is relatively high, and the product can be applied in pig feed production.

Full Text

Nutritional Component Analysis of Saccharomyces cerevisiae Culture Using Distiller' s Grains as Substrate and Evaluation of Its Application Value in Pig Feed

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Abstract

To evaluate the application value of *Saccharomyces cerevisiae* culture using distiller' s grains as substrate in pig feed, this study first determined its nutritional components, including dry matter, crude protein, ether extract, ash, crude fiber, amino acids, and gross energy. The total tract apparent digestibility of nutrients in pigs was measured using the total feces collection method, while the apparent ileal digestibility of amino acids was determined through T-cannula installation. The results showed that the product contained 8.34% moisture, 31.12% crude protein, 5.00% ether extract, 13.46% ash, and 11.00% crude fiber, with a gross energy of 19.53 MJ/kg. Mycotoxin levels were far below national standard limits. The total tract apparent digestibility values for dry matter, gross energy, crude protein, ether extract, crude fiber, and ash were 48.79%, 38.33%, 54.15%, 82.94%, 23.17%, and 20.29%, respectively, yielding a digestible energy of 11.75 MJ/kg. The average apparent ileal digestibility of essential and non-essential

amino acids was 72.38% and 69.37%, respectively, with both lysine and methionine exceeding 80%. These findings demonstrate that amino acids in *S. cerevisiae* culture using distiller' s grains as substrate are highly absorbable and utilizable, making it suitable for application in pig feed production.

Keywords: amino acids; distiller' s grains; *Saccharomyces cerevisiae* culture; nutritional value evaluation; pigs

Introduction

The rapid development of China' s livestock industry has led to enormous consumption of feed ingredients. In 2015, China' s pig production reached 708 million head, consuming 83.5 million tons of feed. Based on existing cultivated land area and production capacity, feed ingredient supply cannot meet current industry demand in the short term, creating a "grain-for-feed" competition with human food consumption. While wheat and rice production remain relatively abundant, corn and soybeans—essential for the most common corn-soybean meal formulations—are severely deficient. Although importing soybeans and corn can partially alleviate this gap, the increasing homogeneity in global grain production poses strategic challenges for securing China's feed grain supply. To address this risk, it is imperative to actively expand feed ingredient sources by incorporating by-products from food processing, herbal medicine production, and fermentation industries into conventional feed ingredient catalogs. Utilizing these unconventional materials represents an effective approach to alleviating the current feed ingredient dilemma.

Distiller' s grains are residues remaining after distillation of spirits from grains such as sorghum and corn, retaining most of the protein, fat, calcium, phosphorus, and other nutrients from raw materials, along with abundant fermentation products. In 2015, China' s liquor production reached 12.5713 million tons. Based on the ratio of 10 tons of distiller' s grains per ton of liquor produced via solid-state fermentation, annual distiller's grain output exceeds 100 million tons. Beyond conventional nutrients like protein and fat, distiller's grains contain substantial purines, pyrimidines, and lipid compounds from yeast autolysis, as well as vitamins, enzymes, and organic acids, making them a promising novel feed ingredient for livestock production. However, fresh distiller's grains are difficult to store and transport for extended periods. Processing through fermentation and other techniques can reduce moisture and crude fiber content while increasing relative nutrient concentrations, improving amino acid profiles, and providing highly active cellulase enzymes, thereby enhancing the reuse value of distiller' s grains. Yeast fermentation can fully utilize distiller' s grains, reduce environmental pollution, and improve nutritional value to create new protein feed with greater economic value. In recent years, *S. cerevisiae* culture using distiller' s grains as substrate has emerged as a novel feed ingredient, but its application in pig production remains limited due to insufficient nutritional data. Therefore,

this study aims to assess its nutritional value and application potential in pig feed, providing a theoretical basis for its rational use in pig production and the feed industry.

1.1.1 Experimental Materials

The *S. cerevisiae* culture using distiller's grains as substrate was provided by Anhui Dongfang Xinxin Biological Technology Ltd., Co. The processing technology was as follows: using distiller' s grains from Anhui Gujing Group as raw material, the substrate was first fermented with selected *S. cerevisiae* (2.3$×10^{8}$ CFU/g) to increase pH for optimal *Bacillus subtilis* growth, then fermented with *B. subtilis* (2.8$×10^{9}$ CFU/g) to form the yeast culture. After fermentation, the product underwent low-temperature drying and rice husk separation to obtain a functional feed rich in nutrients. Twelve crossbred castrated male pigs (body weight 20.0$±$2.0 kg) were purchased from Hunan New Wufeng Co., Ltd., Yong' an Branch.

1.1.2 Experimental Instruments

Equipment included a multifunctional microplate reader (Infinite M200 PRO, TECAN, Switzerland), UV-visible spectrophotometer (UV-2450, Shimadzu, Japan), muffle furnace (C450, Beijing Yongguangming Medical Instrument Co., Ltd.), automatic Soxhlet extractor (SOX416, Gerhardt, Germany), automatic fiber analyzer (FT12, Gerhardt, Germany), isothermal automatic calorimeter (5E-AC8018, Changsha Kaide Measurement & Control Instrument Co., Ltd.), and automatic amino acid analyzer (L-8800, Hitachi, Japan).

1.2 Nutritional Component Analysis of *S. cerevisiae* Culture

Dry matter, crude protein, ether extract, crude fiber, and ash contents were determined according to Chinese National Standards GB/T 6435-2006, GB/T 6432-1994, GB/T 6433-2006, GB/T 6434-2006, and GB/T 6438-2007, respectively. Gross energy was measured using an isothermal automatic calorimeter following international standard ISO 9831:1998. Amino acid composition was analyzed according to GB/T 18246-2000: samples were hydrolyzed with 6 mol/L HCl at 110°C for 24 h, filtered, and analyzed using an automatic amino acid analyzer. ELISA kits for zearalenone, T-2 toxin, deoxynivalenol, fumonisin B1, ochratoxin, and aflatoxin were purchased from Shanghai Enzyme-Linked Biotechnology Co., Ltd.

1.3 Determination of Total Tract Apparent Digestibility in Pigs

The animal trial was conducted in September 2016 (12-day period) at the Metabolism Laboratory of the Animal and Crop Experimental Building, Institute of Subtropical Agriculture, Chinese Academy of Sciences. The basal diet

was formulated according to NRC (2012) nutrient requirements for 20–50 kg growing pigs, with composition and nutrient levels shown in Table 1 . The experimental diet consisted of 30% *S. cerevisiae* culture using distiller' s grains as substrate + 70% basal diet. Twelve crossbred castrated male pigs (20.0$±$2.0 kg) were randomly divided into two groups, housed individually, with ad libitum water access at approximately 25°C ambient temperature. After a 4-day adaptation period feeding the basal diet, the formal trial consisted of a 4-day preliminary period and a 4-day fecal collection period. During collection, pigs were fed 90% of their pre-trial intake twice daily at 12-hour intervals, with total feces collected over 48 h and stored at -20°C. After collection, all fecal samples were thoroughly mixed, and 50 g subsamples were treated with 10 mL of 10% HCl for nitrogen fixation, dried at 65°C to constant weight, ground through a 60-mesh sieve, and stored for analysis.

The total tract apparent digestibility of nutrients in the test ingredient was calculated as follows:

$$D = \frac{A - B}{F} \times 100 + B$$

where:
- D = total tract apparent digestibility of a nutrient in the test ingredient (%)
- A = total tract apparent digestibility of the nutrient in the experimental diet (%)
- B = total tract apparent digestibility of the nutrient in the basal diet (%)
- F = proportion of the nutrient from the test ingredient relative to the experimental diet (%)

1.4 Determination of Apparent Ileal Digestibility of Amino Acids

Six crossbred castrated male pigs fitted with T-cannulas were used to determine apparent ileal digestibility of amino acids using titanium dioxide (0.2%) as an indicator. The experimental diet composition and nutrient levels are shown in Table 2 . The formal trial lasted 7 days, with 4 days for diet adaptation and 3 days for digesta collection. Pigs had ad libitum access to feed and water, fed at 08:30 and 16:30 daily with equal portions. During collection, digesta was continuously collected for 10 h starting from the first feeding, immediately stored at -20°C, then thawed, pooled per pig, freeze-dried, ground through a 60-mesh sieve, and stored at -20°C for analysis.

Apparent ileal digestibility of amino acids was calculated as:

$$\text{Amino acid AID (\%)} = \left[1 - \frac{\text{Amino acid in digesta} \times \text{Indicator in diet}}{\text{Indicator in digesta} \times \text{Amino acid in diet}}\right] \times 100$$

1.5 Statistical Analysis

Experimental data were analyzed using one-way ANOVA with the general linear model procedure in SPSS 19.0 software. Results are expressed as mean ± standard deviation.

2.1 Nutritional Composition of *S. cerevisiae* Culture

The gross energy of *S. cerevisiae* culture using distiller' s grains as substrate was 19.53 MJ/kg, with crude protein, ash, crude fiber, and ether extract contents of 31.12%, 13.46%, 11.00%, and 5.00%, respectively (Table 3). The product showed high gross energy and crude protein content. Derived from residues of sorghum, rice, and corn after liquor production and further fermented with microbial strains, the crude protein content was enhanced to approach that of soybean meal. As shown in Table 4 , the yeast culture had a rich amino acid profile, containing 8.27% lysine, 17.88% leucine, 9.21% alanine, 10.72% valine, 9.99% proline, 6.48% arginine, and 9.16% phenylalanine. The abundant amino acid content and variety can promote animal growth and development.

2.2 Identification of Limiting Amino Acids

In livestock production, essential amino acids must maintain balance with each other and with non-essential amino acids. This study calculated the proportional relationships among essential amino acids in the yeast culture to identify limiting amino acids for pigs (Table 5). Using lysine as the reference (100), methionine showed the lowest ratio at 22, substantially lower than the ideal amino acid ratio for pigs (60). Except for methionine and lysine, all other amino acid ratios exceeded the ideal values. These results indicate that methionine is the first limiting amino acid and lysine is the second limiting amino acid in *S. cerevisiae* culture using distiller' s grains as substrate for pigs.

2.3 Mycotoxin Content

As shown in Table 6 , the yeast culture contained 119.38 μg/kg zearalenone, 15.52 μg/kg T-2 toxin, 542.13 μg/kg deoxynivalenol, 114.26 μg/kg fumonisin B1, 0.09 μg/kg aflatoxin B1, and 0.08 μg/kg ochratoxin—all far below regulatory limits. Aflatoxin and ochratoxin levels were particularly low, while deoxynivalenol was relatively higher among the toxins but still well below the limit standard.

2.4 Total Tract Apparent Digestibility and Digestible Energy

Pigs exhibited total tract apparent digestibility values of 48.79% for dry matter, 54.15% for crude protein, 82.94% for ether extract, 23.17% for crude fiber, 20.29% for ash, and 38.33% for gross energy (Table 7). Ether extract and crude

protein showed high digestibility, while crude fiber and dry matter digestibility were relatively low. The digestible energy of *S. cerevisiae* culture using distiller' s grains as substrate for pigs was 11.75 MJ/kg.

2.5 Apparent Ileal Digestibility of Amino Acids

The apparent ileal digestibility of essential amino acids was generally high, with serine showing the lowest value at 55.10% (Table 8). The first limiting amino acid, methionine, reached 84.92% digestibility, while threonine, valine, and histidine showed values of 71.36%, 70.20%, and 71.13%, respectively. The average apparent ileal digestibility was 72.38% for essential amino acids, 69.37% for non-essential amino acids, and 70.88% for total amino acids.

Discussion

The gross energy (19.53 MJ/kg), crude protein (31.12%), crude fiber (11.00%), and ether extract (5.00%) contents of the yeast culture were determined in this trial. After processing, both gross energy and crude protein contents were higher than those of corn distillers dried grains with solubles (DDGS). Although rice husk separation was performed, some husk residue remained, resulting in 11% crude fiber content. The dehulling process reduced crude fiber content while increasing other nutrient concentrations. Appropriate crude fiber levels can promote intestinal motility, enhance digestive capacity, and improve feed palatability. However, excessive dietary fiber reduces nutrient digestibility. Previous studies found that adding 50% DDGS to dairy cow diets maintained growth performance while preserving protein digestibility and absorption in the intestine. Additionally, DDGS dietary fiber significantly altered colonic microbial characteristics in pigs.

The amino acid profile of *S. cerevisiae* culture using distiller's grains as substrate was rich, containing 8.27% lysine, 17.88% leucine, 9.21% alanine, 10.72% valine, 9.99% proline, and 9.16% phenylalanine, with lysine content higher than that of corn DDGS. During distiller' s grain fermentation, maintaining optimal temperature and humidity for microbial growth poses mycotoxin contamination risks. Major mycotoxins including zearalenone, deoxynivalenol, T-2 toxin, aflatoxin B1, fumonisin B1, and ochratoxin are prevalent and increasingly contaminate raw materials such as corn, barley, sorghum, and wheat used in liquor production. Ochratoxin, zearalenone, and deoxynivalenol are heat-stable and persist through processing. Mycotoxins not only reduce feed nutritional value but also cause gastrointestinal dysfunction, diarrhea, vomiting, and malnutrition in animals. The crude fiber content in fermented distiller' s grains directly affects nutrient absorption and digestibility.

Amino acid digestibility is a critical indicator for evaluating protein nutritional value in monogastric animals. This study determined that *S. cerevisiae* culture using distiller' s grains as substrate exhibits high amino acid digestibility in pigs, supporting its potential as a valuable protein feed ingredient.

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Note: Figure translations are in progress. See original paper for figures.